BRIEFER ARTICLES.

THE CELLOIDIN METHOD WITH HARD TISSUES.

THE following celloidin method, developed and perfected by Dr. E. C. Jeffrey, has been incompletely described at second or third-hand elsewhere,[1] but is here published in full for the first time, in response to numerous inquiries from persons interested in the photomicrography of plant tissues, and in the preparation of large numbers of uniform sections for class use. This method has been employed in the laboratories of plant morphology of Harvard University for two years, and when judiciously applied it is found to leave practically nothing to be desired. The hardest tissues may be cut as thin as $5\,\mu$ or less, without difficulty, if they are first properly treated, and the sections thus obtained are perfectly adapted to photographic requirements. Briefly stated, the method as specialized for the study of wood and other objects containing skeletal tissues, is as follows:

I. *Preparation of material.*—If wood is to be studied, it should be cut up into cubical blocks, in such a way that the faces represent accurate transverse, radial, and tangential sections. The best results are obtained from cubic blocks of not more than 1^{cc}, though much larger ones may be used if the time of treatment be proportionately increased. Material other than wood should be cut in similarly small pieces, and in such a way as to afford the desired plane of section. If the material is dead and dry, the pieces should be repeatedly boiled in water and cooled, in order to remove the air. It is well to pump out the remaining air with a good vacuum pump. In case living material is to be studied, the protoplasm may be killed and fixed by placing the blocks directly in the following solution:

Mercuric chloride, saturated solution, in 30% alcohol - - -	3 parts
Picric acid, saturated solution, in 30% alcohol - - - -	1 part

After twenty-four hours this solution is to be washed out by passing the blocks through alcohols of grades 40, 50, 60, 70, and 80 per cent. Twelve to twenty-four hours should be allowed for each change of alcohol, and the 80 per cent. alcohol should have enough iodin solu-

[1] CHAMBERLAIN, C. J., Methods in plant histology, p. 55; also MILLER, C. H., On embedding in celloidin. Jour. Applied Micr. **6**: 2253–2254. 1903.

tion added to it to keep it of a deep brown color. Moderate heat—50° C.—hastens the process. Chromic acid and chromates, though extremely useful fixing agents for certain kinds of objects, are not at all desirable for lignified tissues, as they are rendered yet harder by the use of these reagents.

II. *Desilicification, etc.*—Woody tissues usually contain more or less silica and other mineral deposits which render sectioning difficult or impossible. Hence it is of primary importance that these substances be removed as thoroughly as possible. For this purpose dilute hydrofluoric acid is indispensable. A 10 per cent. aqueous solution of the commercial acid is most useful. This may be kept in a bottle coated internally with a thick layer of hard paraffin. The blocks of material are transferred directly from the water in which they were boiled, or from the 80 per cent. alcohol and iodin, as the case may be, and are kept in the acid three or four days, with one or two changes of the acid and frequent shaking of the bottle. Wash out the acid thoroughly in running water for two to four hours. This treatment frees the tissues completely of all mineral deposits, while the organic structure remains unaffected. It has been shown[2] that cellulose, constituting the cell-walls of plants, when freed from mineral deposits is of practically uniform hardness, regardless of the plant or part of the plant in which it may occur. This hardness is about equal to that of muscovite, while the tissues in their natural condition often are equal in hardness to calcite, fluorite, or even opal. Hence it is evident that the usefulness of hydrofluoric acid in this connection can scarcely be overestimated.

III. *Dehydration.*—The water must be again removed from the material by passing it through alcohols of grades 30, 50, 70, 90 per cent., and absolute, allowing twelve hours in each grade. At least one change of absolute alcohol is usually desirable, as complete dehydration is of the first importance. The remaining air should be removed from the blocks when they are in 60 or 70 per cent. alcohol, by means of the vacuum pump.

IV. *Infiltration with celloidin.*—Make a series of celloidin solutions by dissolving Schering's celloidin (Schering & Glatz, New York) in equal parts of ether and synthol or absolute alcohol. There should be ten grades: 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 per cent. For the 2 per cent. solution take two grams of celloidin to 100^{cc} of the ether and synthol, or ether and absolute alcohol mixture. For 4 per cent. take

[2] OTT, EMMA, Beiträge zur Kenntniss der Härte vegetabilischer Zellmembranen. Oesterr. Bot. Zeitschr. 50: 237–241. 1900.

four grams to 100^{cc}, and so on, through the series. Synthol (Bausch & Lomb Optical Co., Rochester, N. Y.) is a better solvent of celloidin than is absolute alcohol, and when it is used two more grades of celloidin — 22 and 24 per cent.— may well be used.[2]

Transfer the blocks from absolute alcohol to the 2 per cent. solution celloidin. See that the bottle is almost filled, then wire or clamp the stopper firmly in place, and put the bottle on its side in an ordinary paraffin bath at 50° to 60° C. for twelve or eighteen hours. Then cool the bottle quickly in cold water, taking care that the water does not get into the bottle. Next pour out the 2 per cent. celloidin solution and replace it with the 4 per cent. solution. Proceed in the same way with this and all the following grades of celloidin, up to the most concentrated, and when this grade is reached it is to be gradually thickened by adding a few chips of dry celloidin from time to time, until the mixture in the bottle is quite stiff and firm. With a pair of forceps the blocks are now to be pulled out of the bottle, each with a coat of celloidin adhering, and are to be put into a bottle of chloroform to harden for twelve hours. From this they are transferred to a mixture of equal parts of 95 per cent. alcohol and glycerin, where they should remain for a few days before cutting, and where they will keep for an indefinite period. The time given to the process of infiltration should in no case be shortened, except for very small objects. An increase of the time is often advantageous.

V. *Sectioning.*—The sliding type of microtome is a necessity in the cutting of celloidin material. The best pattern is the Thoma, as recently adapted, at the suggestion of Dr. Jeffrey, for cutting hard tissues. The knife should be extremely hard, as supplied for this purpose by Jung of Heidelberg, and should be kept honed to a keen, smooth edge. It is best to grind and hone the knife with a back, in order that the edge may not be too thin. The blade of the knife should have a dip of about ten degrees in the holder, and should be set more or less obliquely to the line of motion, according to the nature of the material, so as to make a long sliding cut. All bearing surfaces should be thoroughly cleaned with ether and then flooded with the best quality of oil each time the microtome is set up for use. The block of material, if very hard or large, is to be clamped in a special object-carrier (as supplied by Jung), in such a way as to give the desired plane of section. Smaller and softer objects may be cut on the ordinary

[2] The celloidin solutions may be used repeatedly, and should be kept in bottles lying on their sides, to prevent excessive evaporation and concentration of the solutions.

object-carrier. The object-holder in the latter instance consists of a tube of thin metal, plugged with hard wood. The end of this wooden plug is thoroughly coated with celloidin by dipping it repeatedly in 4 per cent. celloidin and drying in the paraffin bath. The block of material is attached to this holder by means of a drop of the 4 per cent. celloidin. One face of the block must be trimmed flat and freed from glycerin before applying it to the moistened surface of the holder. It will set firmly in a few minutes. Run the carrier slowly up the ways by means of the feed-screw, cutting off the celloidin and trimming the block to the desired level. Do not cut thick pieces from the block with the microtome knife, as the latter is very apt to be injured in this way. Adjust the feed-mechanism to give the desired thickness, and then with a camel's-hair brush flood the knife with 90 per cent. alcohol, and, holding the brush lightly on the top of the object, float the section out upon the knife. A slow, steady stroke of the knife generally gives the best results. The section is then removed from the knife by means of the brush, and is to be kept in 90 per cent. alcohol until needed for the next step in the process. The thickness of the section must depend upon the purpose for which the sections are made. For ordinary study with the microscope, 10μ is usually quite thin enough, but for photomicrography it is often desirable to have sections as thin as 5μ or even less.

VI. *Staining and mounting.*—To prepare the sections for staining and mounting, it is usually most convenient to remove the celloidin at once by placing the sections for ten or fifteen minutes in ether, then wash in 95 per cent. alcohol, after which they may be transferred to water, stained, cleared, and mounted in the usual way. One of the most useful stains is the haematoxylin-safranin double stain. First stain the sections to a fairly dense purple in an aqueous solution of Erlich's haematoxylin; wash in dilute aqueous solution of calcium or sodium carbonate, and then in two changes of distilled water. Add a few drops of alcoholic solution of equal parts of Grübler's alcoholic and aqueous safranin, and stain to a rich red. A fairly dilute stain acting for one or two hours will give better results than a more concentrated stain acting for a shorter time. Transfer the sections directly to absolute alcohol, dehydrate rapidly, and transfer to xylol, benzol, or chloroform, and mount in Canada balsam (which has been previously thoroughly dried) dissolved in xylol, benzol, or chloroform. Sections should be cleared in the same kind of liquid as is used for dissolving the balsam in which they are to be mounted.

An extremely satisfactory stain for photographic purposes is the iron-alum haematoxylin of Heidenhain. The method with this stain is given in full by Chamberlain.[3] We find it desirable to wash the sections repeatedly in distilled water after using the iron-alum, before placing them in the haematoxylin. This stain is especially useful in the study of wood sections. A contrast stain of safranin may be added if desired, but it is of doubtful value for practical purposes.

It frequently happens, as in sectioning buds, ovaries, etc., that it is necessary to preserve the celloidin matrix of the section in order to prevent displacement of the otherwise separate parts of the section. In this case the sections are transferred directly from 90 per cent. alcohol to water, and are stained as usual. Dehydrate in a mixture of absolute alcohol and chloroform, clear and mount. The chloroform counteracts the solvent action of the absolute alcohol, and preserves the celloidin film perfectly.

VII. *Serial sectioning.*—In order to make serial mounts by the celloidin method, the sections are cut in the following mixture, instead of in 90 per cent. alcohol: 90 per cent. alcohol 85 parts, glycerin 15 parts. As the sections are cut they are to be arranged on a piece of smooth, thin paper. As soon as the alcohol has evaporated from the sections, turn the slip of paper face down upon a slide which has been coated with albumen fixative, add several layers of paper, and press the whole firmly down upon the slide by means of a photographic squeegee roller; then put another slide on top of the layers of paper, clamp all together by suitable spring clips, and place in the paraffin bath to dry for not more than twelve hours. A longer time than this renders the celloidin more or less insoluble. The paper may now be stripped off, leaving the sections firmly attached to the slide. Pass the slide through alcohol, ether, alcohol, stain, etc., as with separate sections.

The most important steps in this method are desilicification and dehydration of the material. With due attention to these points, and with a proper allowance of time for infiltration, the hardest tissues may be put in perfect condition for sectioning.

The method is found to be of special value wherever it is desirable to reproduce with absolute fidelity by means of photomicrography the appearance of skeletal and other hard tissues of plants. The greatest usefulness of the method, however, is in connection with the teaching of morphology and histology, insuring as it does the absolute uni-

[3] CHAMBERLAIN, C. J., Methods in plant histology, p. 38.

formity of sections for the entire class, thus greatly facilitating the work of instruction, and conserving the energy of both instructor and student.—AMON B. PLOWMAN, *Harvard University.*

ANATOMICAL NOTES ON CERTAIN STRAND PLANTS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LVIII.

THE following notes embody the results of a comparative study of the leaf anatomy of certain plants occurring on the Atlantic coast in the vicinity of Woods Hole, Mass., and also near Lake Michigan in the vicinity of Chicago, Ill. Several of the plants are typical strand plants, *e. g.*, *Cakile americana;* others frequently or even generally occur at a greater distance from the shore. A similar study ot French strand plants has been made by Lesage,[1] and the results recorded in the present paper largely confirm those of the earlier writer.

1. Plants growing in the maritime situation are found to have thicker leaves than the same species growing inland.

Plants	Thickness of leaf in mm.	
	Inland	Maritime
Cakile americana	0.76	1.17
Lathyrus maritimus	0.28	0.32
Euphorbia polygonifolia	0.28	0.38
Xanthium canadense	0.39	0.60
Atriplex hastata	0.19	0.49
Hibiscus Moscheutos	0.13	0.23
Convolvulus sepium	0.24	0.31
Solanum nigrum	0.31	0.37
Polygonum aviculare	0.15	0.27

The foregoing measurements represent average thickness of different leaves and different parts of leaves. Since the Lake Michigan specimens were collected in June and the Woods Hole specimens in July and August, it was thought that the difference in time of collection of the inland and maritime material might introduce a source of error. Accordingly additional specimens of several species were secured in the Lake Michigan region in October, and these yielded the same measurements as those collected in June. It will be seen from the table that in some cases the maritime form is only slightly thicker than the inland form, while in other cases the former is nearly twice as thick. Measure-

[1] Rev. Gén. Bot. **2**: 55. 1890.